ERA-NET NEURON Cofund

Symposium

‘Neurodevelopmental disorders’

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Virtual meeting

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Inserm, France

This Foresight symposium is part of the Work Package

“Outreach and interaction activities”

Work Package leaders are Inserm and CNRS

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Welcome

Dr. Marlies Dorlöchter (DLR-PT, NEURON coordinator, Bonn, Germany)

The coordinator of ERA-NET NEURON, Marlies Dorlöchter, introduced this foresight symposium on 'Neurodevelopmental disorders' by welcome addressing all attendants: scientific speakers, representatives of patient organizations, members of the NEURON Scientific Advisory Board, and representatives of NEURON partner organizations. She highlighted the value of this meeting for funding organizations to understand the key aspects in the field of neurodevelopmental disorders.

ERA-NET are networks of funding agencies and ministries, in Europe and beyond, getting support from the European Commission. NEURON (Network of European funding for Neuroscience research) is an ERA-NET in the area of brain research. Starting in 2003 with four funding organization it developed constantly and comprises today 27 funding organizations with partners well beyond Europe such as Canada and Taiwan. One of the key elements of NEURON is launching Joint Transnational Calls (JTCs) for research proposals, because multilateral, interdisciplinary innovative research is key to explore the brain and its diseases, and to help finding therapies and diagnosis tools for various disorders. A special feature of NEURON are the calls for proposals for research projects on Ethical, Legal, and Social Aspects (ELSA) of Neuroscience, a unique international funding instrument. NEURON's purpose as a network is not only to promote brain research, but also to improve interactions between the research community, policy makers, funding organizations and the general public. In discussions with European and national policy makers NEURON strives to gain enhanced consideration for brain research. It also interacts with the research community in various formats such as foresight symposia, workshops, newsletters and journal editorials. NEURON also includes programs to support early-carrier researchers such as inviting them to networking activities and FENS conferences as well as the Excellent Paper in Neuroscience Award.
These activities are based on a research strategy: world experts from various scientific fields and from the NEURON Scientific Advisory Board developed a Scientific Research Agenda (SRA). It was first published in 2016 and recently updated in 2020. The SRA covers the entire field of brain diseases: brain research on neurological diseases, psychiatric disorders, sensory organs diseases and peripheral nervous system disorders. The priorities covered in NEURON calls and other activities are to understand diseases mechanisms, to understand disease progression, and develop interventions. In the process of publishing SRA, the research communities (namely professional societies and patient organizations) were also asked for their input on this research agenda, and NEURON can comfortably rely on 80% approval or even entire acceptance.

ERA-NET NEURON has spent over 136 million euros in funding research projects with so far 14 Joint Transnational Calls for research proposals. Interdisciplinary and multilateral research is funded *via* small to medium transnational research consortia (a maximum of 6 research groups from at least 3 countries). The ERA-NET NEURON participants commonly decide for the individual topics and embodiments of the JTCs that have to match both, the national strategies and research communities and urgent scientific needs.

Introduction

Dr. Etienne Hirsch (INSERM, Paris, France) and Dr. Bernard Poulain (CNRS, Paris, France)

Etienne Hirsch and Bernard Poulain presented the general objectives of this foresight symposium. Its purpose it to debate on a very hot topic in order to prepare the next call focusing on neurodevelopmental disorders (NDDs). Neurodevelopment in this context is defined as all processes involved in building a brain and nervous system that is functional, not only in that network and motion work properly but also regarding cognitive aspects. In human, to build a fully functional brain requires at least 25 years, including embryogenesis, foetal stages, childhood and adolescence. In this definition can also be included development and learning, as the brain has to be trained in order to work properly. During these 25 years of brain and nervous system development, there are multiple critical windows of time during which neurodevelopment may shift aside from its normal trajectory, which constitutes the main cause of the different disorders we are to address today.
This symposium has several general objectives:

- To provide an overview of the major NDDs
- To review normal development of the nervous system
- To review the genetic background of NDDs
- To present a focus on ASD and childhood epilepsy syndromes
- To address the burden of NDDs and intervention strategies
- To discuss about a possible call focused on NDD research

To cover these aspects, experts have been invited to give their professional advice on preparing the next joint transnational call on neurodevelopmental disorders.

Nervous system development

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The expansion of the neocortex is one of the major features of nervous system development in humans, and a hallmark of hominid evolution. Specifically, there has been a steep increase in neocortex size and folding, two distinctive features of the human brain, that started approximatively 3 million years ago. Hence, it is a major challenge to elucidate the genomic and molecular basis of neocortex expansion and folding.

It has been shown that neocortex expansion is based on the abundance and proliferative capacity of neural progenitor cells during development. The so-called apical progenitors, which reside in the primary germinal zone, the ventricular zone, are the first cell type involved. These progenitor cells can undergo mitosis only at the ventricle, which is a very limited space. To overcome this limitation for maximizing neural progenitor mitoses, apical progenitors generate the so-called basal progenitors for the subventricular zone. In species with an expanded neocortex, this secondary germinal zone becomes thicker and thicker, and basal progenitors can undergo mitosis anywhere in the subventricular zone. This allows for an increase in neuron production, a key step toward neocortex expansion. Recent investigations on brain development have revealed some of the genomic basis of neocortex expansion in humans, namely the importance of human-specific genes.

Specifically, the human-specific *ARHGAP11B* gene has been identified to have a pivotal role in neocortex expansion, even though the underlying mechanisms are still being investigated. Animal models have been of great help in these investigations, as shown by the following examples. A first study showed that ectopic expression of *ARHGAP11B* in the neocortex of mouse embryos, animals known for their smooth brain, can result in neocortex folding (Florio *et al*., Science, 2015). However, ferrets are considered better models than rodents regarding brain development and its disorders, as illustrated by an *Aspm* KO ferret model for microcephaly recently developed in the US that showed a strong decrease in brain size. Accordingly, ectopic expression of the *ARHGAP11B* gene in developing ferret neocortex induced (i) an increase in the proliferation of basal progenitors, (ii) a lengthening of the neurogenic period, and (iii) an increase in the number of upper-layer neurons, all hallmarks of neocortex expansion (Kalebic *et al*., eLife, 2018). Finally, *ARHGAP11B* expression in transgenic fetuses of marmoset monkeys established the physiological relevance of these observations, with the neocortex getting bigger and starting to fold during gestation (Heide *et al*., Science, 2020). This last study, which for ethical reasons was confined to the fetal stage, underscores how important transgenic monkeys are for research on brain development.

Dissecting the molecular mechanism underlying this neocortex expansion, *ARHGAP11B* has been shown to act in mitochondria, increasing the activity of a metabolic pathway called glutaminolysis (Namba *et al*., Neuron, 2020). Glutaminolysis is known to be increased in rapidly proliferating cells, such as cancer cells. These findings therefore highlight the importance to study the metabolism of neural progenitors in future research.

To gain further insight into the process of human neocortex expansion, it will be essential to investigate the underlying mechanisms in models more closely related to human. While fetal neocortex tissue can be obtained from human but not from chimpanzee (the closest living species to us) as the latter is a protected animal species, an interesting alternative for models of brain development are 3-dimensional cerebral organoids of human or ape, which can be grown

from induced pluripotent stem cells. Cerebral organoids allow the comparison of chimpanzee vs. human brain development. In a recent study with chimpanzee and human cerebral organoids, it has been uncovered that human apical progenitors spend more time in metaphase during their mitosis than chimpanzee apical progenitors, with the metaphase being 50% longer in human (Mora-Bermúdez *et al*., eLife, 2016). This finding suggests that chromosome distribution may occur with less errors in human apical progenitors, which could be important considering the potential significance of chromosomal abnormalities for brain development. While folding has been reported to occur in cerebral organoids (Karzbrun *et al.*, Nature Phys, 2018), this key process of brain development has recently been studied in *ex vivo* cultures of fetal human neocortex tissue obtained from abortion. Specifically, it was shown that addition of three specific extracellular matrix components are sufficient to induce human cortical folding in a hyaluronic acid-dependent manner (Long *et al*., Neuron, 2018).

This overview of the investigation of neocortex expansion has briefly discussed several interesting mechanisms and animal models in the context of research on brain development. It is important, for the sake of future research on neurodevelopmental disorders, to encourage further investigation of these aspects.

General overview of neurodevelopmental disorders

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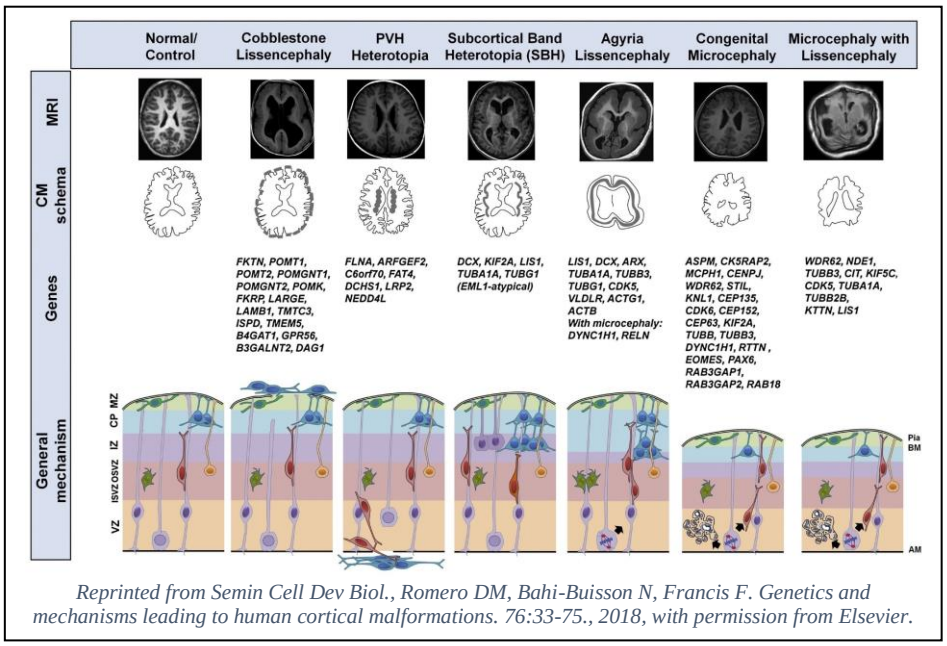
The topic of neurodevelopmental disorders is a wide one that encompass a large array of pathologies. Even reducing the focus to cortical malformations alone, which are associated with epilepsy and intellectual disability, we can distinguish them into several categories mostly based on the underlying neuroanatomical differences caused by various problems of cell production and neuronal migration. Still, there is a lot to investigate, for example to understand the relation between a specific problem of neuronal migration and the possible outcomes.

In this regard, investigations related to genetics findings and in different types of models are of the upmost importance. Multiple models are being combined that provide valuable information, including mouse, drosophila, zebrafish, ferret and human in vitro models (with cells being reprogrammed to generate human cortical progenitors and neurons assembling in 3D structures such as organoids). Research on these disorders faces the issue of the large genetic heterogeneity, with new mutant genes being constantly identified in patients. The required investigation on each gene to identify its impact on cellular mechanisms is a very time and resources demanding process, making it challenging for the neuroscience community. To speed up this investigation work, it is essential to develop new ways to validate mutant genes, namely by fostering consortium approaches and collaborations with experts in different domains. Another topic is raised on how a specific mutation can lead to one of the several phenotypes of cortical malformations. For a number of cortical malformations, some general ubiquitous genes are mutated that affect for example, the cortex as well as the cerebellum and other brain regions, however why these structures in particular are so affected remains unanswered, except for theories about protection in other areas due to different expression patterns and compensatory factors. There is much still be learnt about these processes.

The first phenotype of cortical malformation discussed here is microcephaly, related to the brain being too small. A first mechanism leading to microcephaly resides in abnormal cell death in the developing brain, either of the neural progenitors or of neurons, causing a decrease in brain size. Another, more specific, mechanism involves an alteration of proliferating progenitor cells.



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There is a very fine balance in the regulation of these neural progenitors, as it is important to undergo enough proliferative divisions before starting neurogenesis, a mode where progenitor cells generate either other types of more neurogenic progenitors or neurons. If this balance is disturbed and neurogenesis starts too early, it reduces the number of neuron progenitors, hence ultimately decreasing the number of neurons in the brain that ends up being too small.
Several mutant genes have been found to be associated with this disorder, for which cellular investigations previously linked mostly to the centrosome, but more widely some have also been shown to be associated with transcriptional regulation (MCPH1, CENPJ and CDK5RAP2), cell-cycle progression and checkpoint regulation (MCPH1, CENPJ and CDK5RAP2), centrosome maturation (CDK5RAP2 and CENPJ), DNA repair (MCPH1) or the proliferation capacity of progenitors (ASPM and STIL).
The centrosome, as the microtubule-organizing centre, is an essential organelle in brain development especially during cell division, due to its role at the poles of mitotic spindles. Yet, centrosome perturbation may not be the only pathological mechanism that can cause microcephaly, with over 15 genes identified that either have well-known non-centrosome functions or have no known relation with the centrosome. This is a topic for future work to identify the other pathogenetic mechanisms involved in microcephaly.

Lissencephaly, on the other hand, is recognized as a neuronal migration disorder resulting in abnormal formation of cortical folds. Videomicroscopy of mutant cells has allowed to investigate their migration linked to problems of cortical layering. These misplaced neurons can develop abnormal functions, often still to be identified in models. Studies of mutant genes identified in lissencephaly revealed problems in the microtubule cytoskeleton of migrating neurons, though further methods are required to analyse microtubules in living cells and identify which microtubule compartments are involved.
Importantly, microtubules are also critical for the radial glia progenitor cells, and some mutant genes have been shown to affect these cells. This can hence lead to another mechanism for lissencephaly, as these progenitor cells provide an essential substrate for neuron migration: disrupting these cells can hence also disrupt the normal layering of cortical neurons. These findings stress the importance of studying defects in cell-type specific models, so that investigations focus not only on neurons but also on other cell types that might be affected.
Also, in terms of study methods, as well as knock-out models it is important to investigate individual patient mutations for example in knock-in models, as this is often closer to the situation in human, and it could also help explain phenotype variability in patients. Multiple models may hence be required to help understand the functions of frequently mutated genes.

Periventricular heterotopia is another cortical malformation presenting misplaced neurons, as they accumulate at the apical ventricular surface. While mutant genes remain unidentified in some patients, the most frequently mutated gene is filamin A (FLNA), coding for an actin cytoskeleton binding protein that regulates adhesion components such as integrins. Periventricular heterotopia genes could play important roles in both migrating neurons and progenitor cells, thus distinguishing primary defects in these cell types is central to understanding the pathological mechanisms of this disorder.
Furthermore, recent transcriptomics and proteomics data also demonstrated problems in the extracellular matrix in some mutant conditions. These findings are important for focal and mosaic disorders, as in a mosaic situation such mutant genes could influence even non-mutant adjacent cells. Impacts on the extracellular matrix should also be assessed systematically in cortical disorders, and these data are currently for the most part missing. This could require re-evaluation of existing models.
Finally, it has sometimes been possible to identify mutations in human-specific isoforms, which

constitutes an understudied area for these pathologies. It is important, in this regard, to identify such genes and to consider human or primate gene and protein expression patterns, isoforms and alternative splicing, as well as protein partners that may help explain regional effects and disease mechanisms.

The last cortical malformation disorder mentioned here is cobblestone lissencephaly, which is related to a problem of radial glial cells attaching to the pial basal region of the cortex. One of the key disrupted processes for this disorder relies on abnormal glycosylation of alpha-dystroglycan, a glycoprotein complex located in the membrane at the basal extremity of radial glial cells. This abnormal glycosylation impairs the interaction of the cell with the extracellular matrix and reduces its attachment to the cortical surface. The resulting breakages in the basement membrane allow migrating neurons to move out of the cortex and accumulate at its surface.
It is interesting to note the similarities between cobblestone lissencephaly and polymicrogyria, another pathology characterized by small folds on the surface of the brain. Similar mechanisms of radial glial cell detachment could be involved, such as hinted by mutations of GPR56, a receptor in the membrane of radial glial cells that interacts with the extracellular matrix. Yet, the differences between these phenotypes are not fully understood, nor are the mechanisms producing these multiple small folds, requiring a better understanding of cortical folding.
Lastly, not all the observed defects and phenotypes may be caused by problems in radial glial cells. Recent work showed that some of the mutant genes might have wider functions for example in post-mitotic neurons and synapses, hence investigating these cell types may help explain variable phenotypes.

This overview of cortical malformations shows how, in the current state of research, a lot still remains to be investigated in order to understand the fundamental mechanisms involved in these disorders. Gene discovery is valuable as it can contribute fundamental mechanisms, to also help advance translational research. It is clearly important to develop multiple models, both gyrencephalic and lissencephalic, to study the patient-specific mutations identified and this requires a consortium approach. Some above-mentioned examples of cortical malformations also stressed how essential it is to investigate not only the main but also alternative functions of mutant genes in human cells and model organisms. This, together with omics data, will help identify the perturbed pathways in different cell types, overall contributing to understanding the resulting disorder.
An important topic to foster continued research is neuronal migration, not only considering neurons themselves but also their interactions with other cell types and with the extracellular matrix. This research, to be the most pertinent, also requires refined knock-in, cell-type and stage specific models. Investigating the abnormal functions induced by the misplacement of neurons is also essential, requiring further research groups.
Indeed, these projects require collaborations between clinical, molecular, cellular and physiology laboratories to obtain integrated views on cell dysfunctions. Lastly, cortical folding is another important topic, about which very little is known, and this also requires combined expertise across Europe and beyond.
A large gap still exists between understanding these disease mechanisms and identifying potential therapies for these *in utero* disorders. To date, finding methods to rescue the phenotypes remains an important preoccupation for cortical malformation research.

Genetics of neurodevelopmental disorders, prenatal diagnosis

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Early diagnosis is a key topic in the care strategy for neurodevelopmental disorders. It is also a question on which families have high expectations, hoping for non-invasive blood tests that could detect major genetic diseases and predict healthy babies during or even before pregnancy. Yet, the current diagnosis tools unfortunately remain very far from these expectations.
The closest results achieved in this direction rely on pre-conceptional carrier screening. Genetic investigation in parents allows to identify persons or couples at increased risk of giving birth to a child with the tested autosomal recessive disorders. While its use for diagnosis is only limited, this method provides useful information on the epidemiology of these disorders. In a high-throughput sequencing study on 400 genes, for example, it has been showed that every individual was a carrier of an average of 2.8 autosomal recessive diseases (Bell *et al.*, Sci Transl Med, 2011). Another study from 2019 investigated the frequency of couples being at risk, rather than single individuals. Using data from high-throughput sequencing databases across ethnicities, 415 genes were analysed in 123,000 persons, showing a frequency of risk couples ranging from 0.17% to 2.52% depending on the ethnicity (Guo *et al.*, Genet Med, 2019).

Nevertheless, such estimations only cover but the tip of the iceberg as genetic diagnosis, and carrier screening in particular, faces a strong issue of correct variants classification. Individual genomes include an immense amount of missense variants, most of which are rare variants, with only 2% having an entry in the ClinVar database (and half of those being registered as "Variant of Unknown Significance"). This means recessive disease genes as a whole represent over 2,500 established genes identified with obvious pathogenic variants that can currently be used in prenatal diagnosis, in addition to over 2,500 recessive disease genes that are either "Variant of Unknown Significance" or unrecognized variants, and nearly 17,000 genes with unclear disease association. Additionally, other mutational mechanisms need to be considered, such as epigenetic methylation abnormalities and intronic variants that could not easily be detected with the current routine high-throughput sequencing methods. This is especially important as these mutations are common in our populations, with recessive alleles that require special testing accounting for an estimated 30% of the disease risk.
Finally, about 60% of severe neurodevelopmental disorders are caused by *de novo* variants unpredictable with pre-conceptual carrier screening, thus requiring to test the fetus during pregnancy. Hopefully, extensive experience has been gained in the last decades in prenatal diagnostic of chromosome aberration and copy-number changes, allowing to use conventional carrier typing and chromosal microarray testing in both low and high risk pregnancy to identify potential chromosomic disorders. Still, there is a difficulty on prenatal high throughput sequencing, in that prenatal gene-panel sequencing appears to be more efficient in some fetal phenotypes than others (Lord *et al.*, The Lancet, 2019). Thus remains the major issue of variants interpretation, which causes both a diagnostic dilemma and a research problem.
Testing the fetus during pregnancy has however proven itself important even for easily recognized *de novo* variants, and especially for diseases causing genotype-phenotype correlations. In the case of the SCN2A gene, for example, omission variants can cause different phenotypes that are difficult to predict from variant position, and one third of the known diseases genes are similarly associated with more than one phenotype. This poses a problem of medical incertitude when a mutation is identified that is known not to cause the disease in every carrier, which is stressful for families and can hinder their informed decision.

With the currents tools and knowledge in genome interpretation, we are still far from the

efficient non-invasive whole genome analysis of sequence variants that families expect. Although a more advanced proof of concept study was published in 2012, the current state-of-the-art non-invasive prenatal testing is limited to identification of the 3 common trisomy in most countries, with some more advanced laboratories offering non-invasive copy-number profiling test.

In summary, improving the prevention of neurodevelopmental disorders by means of carrier screening and prenatal testing requires further diseases genes identification and to improve variants interpretation and prediction of phenotype caused by a given genotype. It would also be valuable to foster techniques improvements in genome sequencing for both detection of special mutation types and more efficient non-invasive prenatal testing. This means developing sequencing techniques and mapping algorithms but also high throughput functional testing and tools to predict functional impairments.

Besides the monogenic pathogenic variants, it is also important to bring focus on some understudied topics. Namely, non-polygenic risk factors and protective variants affecting the disease risk and severity are important to consider, as the lack of information on natural history and modifying factors contributes to diagnosis uncertainty.

Focus on autism spectrum disorders

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Evidence suggest that several neurodevelopmental disorders are likely to share common mechanisms; such evidence include the fact that a same determinant can lead to different neurodevelopmental disorders and the fact that for most neurodevelopmental disorders males are more affected than females. To understand these commonalities, scientists have investigated in depth the genetics of neurodevelopmental disorders,
Autism Spectrum Disorder (ASD), notably, is known to have very strong genetic determinants, with over 1,000 genes identified that are potentially involved in autism. Investigation of these genes showed that about one half were associated with proteins located in the synaptic cleft, either presynaptic or postsynaptic. Yet, beyond genetics, environmental factors have also been shown, both in human and animal studies, to be of particular importance.
So is also epigenetics, either playing a direct role or being a consequence of other factors, as suggested by changes in epigenetic marks (notably, but not exclusively, methylation) observed in post-mortem studies in human and animal model studies. A recent study went further in correlating changes in transcriptome with patients' ASD clinical scores, using single cell RNA sequencing in the brain of ASD patients (Velmeshev et al., Science, 2019). These results pinpointed how changes the most associated with clinical signs happened in the transcriptome of specific cellular types, namely projecting neurons from upper layers, interneurons and microglia. This proves interesting as microglial cells have been identified in the last decades as playing a major role in brain development, especially in the regulation of the number of synapses and therefore connectivity. These results thus further highlight the importance of microglia in autism, as changes in microglial transcriptome could lead to abnormal connectivity in the brain.

The current increase in incidence of ASD raises some important questions and could be linked to the exposure to more and more environmental factors that might prove toxic for the developing brain. Many factors from the environment have indeed been shown to be associated with autism, with the most significant one being prematurity. About 10% babies are born preterm around the world and cognitive impairment is a main consequence of prematurity, with higher risks of low IQ for more preterm babies. Yet, it has also been shown that preterm infants can have up to 7 times higher risk of ASD compared to term infants. This increased risk from prematurity can even build up with other risks factors, leading to major risk situations. Sex, for example, is an important factor for ASD as in many other neurodevelopmental disorders, with boys being more affected than girls.
Inflammation is also to consider, as the risk to develop autism is 16 times higher in preterm compared to term babies in the context of chorioamnionitis (intra-amniotic infection). This constitutes a major environmental risk factor for autism, especially considering that preterm infants are at higher risk of amniotic and inflammation in utero and systemic inflammation after birth. In itself, inflammation is believed to increase the level of inflammatory cytokines in blood, activating microglial cells, as suggested by animal model studies: systemic inflammation disrupts many genes, leading to inflammatory microglia that can be toxic for developing neural cells. Moreover, inflammation likely affects the timepoints in development, which are normal changes of gene expression in microglia over development. With very few of these genes still being modulated in the context of inflammation, it appears as a double hit condition where microglia both stop its normal function in brain development and become inflamed.
This is further stressed by a recent transcriptomic analysis that showed gene expression

modulation in microglia in preterm infants (Krishnan *et al*, Nat Comm, 2017). Not only are "master genes" affected that play a role in activating microglia but also genes supposed to be expressed in synapses, such as DLG4 or SHANK. Hence, microglial cells express synaptic genes during brain development before neurons do so, and we know these genes are important in regards with genetic factors of autism. This all suggests that not only neurons but also microglia can be affected by mutation of those genes, making the interplay between these cell populations important to investigate in these genetic conditions. It seems clear now that microglia is particularly important in the context of autism and other neurodevelopmental disorders and efforts should be fostered in this direction.
Interneurons, also, are important during brain development for controlling the windows of plasticity of excitatory neurons. It has been shown in the past that their transcriptome is affected in autism and more recent studies in both ASD and premature infants pointed out selective defects in specific subgroups of interneurons (Zikopoulos & Barbas, Frontiers Hum Neurosci, 2013; Stolp et al., Front Physiol, 2019).

Overall, these findings suggest to foster research efforts on better understanding the interplays between environmental and genetic factors, and some cell types seem to be particularly important in this regard. Microglia, as explained earlier, are key cells to be investigated in autism and neurodevelopmental disorders, and so are astrocytes and interneurons for their role in brain development. These interplays, especially when considering inflammation and prematurity, also raise questions regarding other interactions, such as the role of microbiota and gut dysfunction in these disorders.
It is also important to consider disorders over lifetime, as changes in phenotype of ASD patients might appears during adolescence. Observations suggest that some unknown mechanisms of resilience exist in some patients, and understanding these could significantly improve the condition of other patients whose phenotype did not improve.

Childhood epilepsy

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Affecting about 1 million children and adolescents in Europe, childhood epilepsy is an important topic to address, with over 130,000 new patients diagnosed every year. Despite initiatives to improve diagnosis and treatments, such as the European Reference Network Epi-CARE, this disorder remains difficult to tackle as it is a non-static disease, evolving through a process called epileptogenesis from a single first seizure into chronic epilepsy, and a lot remains to understand about epileptogenesis. Namely, while it can be possible to identify children at risk on the basis of some genetic changes or brain damages, it is very difficult to predict the appearance of the first seizure, the evolution into chronic epilepsy, or a patient evolution as clinically refractory. In addition, spontaneous remissions may happen in childhood epilepsy, implying that a point of no return could exist over the course of epileptogenesis, yet the underlying mechanisms remain unknown. Thus, the current lack of measures for individual prognosis as well as the lack of understanding of the interplays between seizures, treatments, cognition, and brain development make childhood epilepsy especially difficult to manage for clinicians and an important burden for patients and families.

There is still a long way to go in treating childhood epilepsy, considering that about 30% of patients are considered difficult to treat, consequently experiencing increased educational difficulties and mortality and overall reduced quality of life. This number has remained mostly unchanged in the last two decades, with most treatments focusing on suppressing the seizures, rather than treating epilepsy as a disease. Yet, a few interesting approaches are to be investigated that step in this direction.
In the last decade, some studies have been following the path of disease-modifying drugs, focusing on epileptogenesis and trying to treat and prevent it. Namely, the EPISTOP study (from 2013 to 2018) investigated how Vigabatrin administration prior to the first seizure could decrease the severity of epilepsy by inhibiting the breakdown of GABA, allowing to start treating patients before the seizure even happens. Other studies highlighted how the mTORC1 pathway could prove important for future treatments as it affects the epileptogenesis mechanisms, and disease-modifying treatments in Tuberous Sclerosis Complex and focal epilepsy are already being investigated. Another path toward these disease-modifying interventions relies on gene therapy for epilepsy, namely targeting monogenetic diseases, although some mutations could prove too large to address. Another envisioned solution could be to target specific epileptogenesis mechanisms (using, for example, antisense therapy), thus allowing to prevent epilepsy or reduce its severity.
A second approach focuses on neuroinflammation-associated epilepsies, such as Rasmussen, Autoimmune encephalitis and FIRE/NORSE. The increasing knowledge about the mechanisms of neuroinflammation allows to build more understanding on its potential role beyond these specific aetiologies, namely on perpetuating further seizures, on cognitive disability and on comorbidities. This new knowledge may help shape new avenues for treatments, as suggested by recent evidences that intravenous administration of immunoglobulin could improve seizure activities, even in patients with focal structural epilepsy (Al Amrani *et al*., Pediatr Neurol, 2017).
Other studies rather focus on developing targeted therapies for the causes of epilepsy, such as the many genes newly identified in epileptic encephalopathies. Clinical trials have been attempted in line with this approach to treat KCNT1-related epilepsy (Fitzgerald *et al*.,

Neurotherapeutics, 2019) or to use sodium channel blockers as a treatment for SCN8A-related disorders (Gardella *et al*., Epilepsia, 2020). Yet, even with targeted therapies, treatment responses predictability remains an issue. Why some patients respond very well while others do not profit at all from the treatment is a broad and ununderstood question that really need further investigation. Future epilepsy treatments would thus hugely benefit from the development of new platforms, namely on genetic zebrafish models, to test these for individual mutations and to develop even better targeted therapies.

Lastly, there is an important problem to address in that the current treatments for childhood epilepsy are not tailored for the developing brain. Notably, it is known that the neurotransmitter situation changes rapidly in the brain during the first four weeks of life, raising questions on how to deal with neonatal seizures, as it is very unsure whether it is preferable to treat these seizures or to hold the treatment to avoid damages in the long run. As an example, Levericacetam has been used for over 10 years for neonatal seizures, even though a clinical trial proved Phenobarbital to be more effective, as the community has been worried about the risk of apoptosis in neonates treated with Phenobarbital. There is a very limited understanding on the long-term effects of these drugs on a developing brain, which prove difficult to correctly treat these patients.
Even though it is critical to investigate the underlying mechanisms and to improve trial design for these treatments, testing a drug in the paediatric population yet remains a major issue and the validity of extrapolating from adult data is very debatable. Whenever it is possible to conduct trials in the paediatric population, another problem arises in the difficulty of recruiting these patients. This also worsen the already important issue of stratification, with different aetiologies of epilepsy often being grouped together due to the limited number of patients available. While the stratification issue also exists in adult, it is more challenging in the paediatric population as there are much more similar aetiologies in adults that in children.
Rodent animal models could be envisioned for treatment investigation, as they have already been used in the context of classical analysis for temporal lobe epilepsy to study anti-epileptic drugs and epileptogenesis, but they appear to be more suitable for adult aetiologies. There is an increasing demand however for genetic models, such as rodents and zebrafish, that proved helpful when it comes to some specific epilepsies and encephalopathies. Rare diseases networks are already being acting in this direction, with patient going toward these networks to model their exact mutation in order to test medication, which will provide useful big data in the long term.

There are also big challenges in epilepsy regarding diagnostic and how to improve it. A significant issue is that cued epileptic spikes are still used for diagnosis, which is not well understood but has since been shown to be neither sensitive nor specific. Meanwhile, improvements in clinical neurophysiology have provided more electrodes and recording sequences, allowing to investigate a larger frequency spectrum of events in diagnosis. As an example, using high-frequency oscillations could help identify if a patient is going to develop epilepsy following the first seizure, as it highlights individual differences between patients with epilepsy and patients with only a single seizure, while the above-mentioned cued spikes does not. Thus, one can hope that these technological progresses will allow for more individual prognostication, using new biomarkers for EEG, MRI and other diagnostic tools.
Another important gap in knowledge to address is related to quality of life and support for patients and families. Comorbidities in paediatric epilepsy is less understood that in adults, in regards to sleep, behaviour and mental health. This represents a heavy burden for families as these kids are not just “small adults”. Currently used disease education and wearables (i.e. to detect and record seizures) might not be appropriate, with the example of people testifying that

education on “sudden unexpected death in epilepsy” brings anxiety. It is very important to foster dialogue with patients’ and families’ representatives so that it is possible to ask for their needs and adjust to it.

In conclusion, the future of paediatric epilepsy research relies heavily on understanding the mechanisms and natural course of epilepsy and epileptogenesis, in order to move from suppressing seizures toward disease-modifying treatments. On the other hand, further focus is required on investigating comorbidities in children epilepsy and working closely with patients and families to address their real needs, thus improving their quality of life.

Patients care, epidemiological studies, socio economics

Dr. Tony Lloyd
CEO of ADHD Foundation, Liverpool, UK

Continuous efforts have been made in the past decades to better identify and diagnose neurodevelopmental disorders and, although a lot remains to be done, it allowed to have a better idea of how high is their prevalence. An estimated one in five people in the general population is potentially affected by one or more such disorder. In the UK, about 15% of the school-aged population is diagnosed with some form of neurodevelopmental condition, without even accounting for the large proportion of undiagnosed patients, as an estimated 40% of children with neurodevelopmental condition are not identified before age 16. Yet, even children and adults correctly diagnosed are too often seen through a unique diagnosis lens, considering one condition rather than the broader neurodevelopmental spectrum. This is important considering how common comorbidities are in neurodevelopmental disorders: as examples, up to 4 in 5 patients with autism spectrum disorder have co-occurring developmental coordination disorder, and 1 in 2 people with dyslexia also has dyscalculia (Cleaton & Kirby, J Child Dev Disord, 2018). This constitutes a clear issue and raises several questions on how to care for those patients, especially regarding education of children with neurodevelopmental disorders.

Some insightful lessons can be learnt on this regards with the example of Attention Deficit Hyperactivity Disorder (ADHD). The global prevalence for this disorder is of one people out 20 in the general population, ranging from 2,5% for adults to 5,9% in children (Willcutt, Neurotherapeutics, 2012). Yet in UK, where one student in every class in average is affected by ADHD, it remains largely underdiagnosed and many confusions are made in education and social care for this disorders.
That is to say, being a multifactorial disorder caused by both polygenic genetic factors and environmental influence, a lot remains to understand about ADHD. It is known, for example, to have strong physical and mental impacts across the lifespan, correlating with increased risks of obesity, diabetes, eating and sleeping disorders and epilepsy. Yet, it is not fully understood what causes these comorbidities nor how to treat it accordingly. Diagnosis is another large hurdle, as stated earlier, and can be proved even more so when investigating the gender ratio in ADHD. On average, there are twice as many males as females affected by ADHD, yet evidences suggest this could be a diagnosis bias: as girls tend to show fewer disruptive behaviours, the disorder is more difficult to recognize. This further delay the diagnosis in females, impacting their life chances by increasing the risk of anxiety and mental distress during childhood and predisposing them for mental health condition later in life.
In terms of treatments as well, many efforts remain to be made to better manage ADHD. Pharmacological interventions, despite their low effect size, are still the recommended first line of treatments as non-pharmacological interventions have demonstrated even lower effects. In these circumstances, only multi-modal treatments, as a combination of different types of intervention, can really be useful to help manage this disorder.

This situation led charities in the UK to face the government and National Health Service to seek explanations on these many health inequalities that remain on ADHD, a very studied disorder, even though some could be inexpensive to improve. asking for more recognition from politics. It is a fact that ADHD is undiagnosed and unmanaged in many adults in UK, with half of the newly diagnosed adults being parents consulting as a result of their children going through the diagnosis process. Studies in Denmark proved late-diagnosed ADHD to be very costly, inducing for an adult around €8,600 additional private costs per person and per year, and around €9,000 public costs (Daley *et al.*, Oxford Press, 2015). These costs mostly result from

lower incomes, as ADHD correlates with poor mental health outcomes, poor socioeconomics outcomes, premature mortality, and detrimental life changes, as illustrated by the 25% of the present prison population in UK that meet diagnostic criteria for ADHD (Young *et al.*, BMC Psychiatry, 2018).
Charities pointed impairing professional boundaries, with ADHD being seen as a behavioural disorder that belongs solely to the field of psychiatry. The fact that research and best practices do not find their way through primary care and children workforce contributes to the lack of information and widespread misunderstanding about ADHD in the daily environment of potentially affected children. This highlights an important need for more communication between the scientific community and the care workers, especially general practitioners.
These claims were heard in the UK and led to the construction of a Multidisciplinary Strategy group, comprised of patient groups, clinicians, researchers, pharmacists and public health executives. Among their important recommendations is the fact that ADHD should not be set in psychiatry alone but should have an integrated multidisciplinary approach, moving the identification, diagnosis and daily management process to primary settings for earlier diagnosis, and tasking psychiatrists to manage complex comorbidities. They proposed to use new technologies for better diagnosis, monitoring and treatment options. In diagnosis, this means moving from observational questionnaires toward computer-based quantitative cognitive functioning tests, with different school and clinical versions so early identification of ADHD profiles in school can be referred to a specialist clinician and undertake a Quantified Behavioural test. This also means developing psycho-education training for parents and school workers and the development of psycho-education, self-care and self-management tools for kids (such as smartphone applications with remote patient monitoring). Lastly, it has been recommended to plan public health campaigns to avoid stigmatization and to inform decision makers of the hidden costs of undiagnosed and unmanaged ADHD.

It is important to consider that, with 1 in 10 people affected by dyslexia, 1 in 20 by ADHD, 1 in 100 by ASD, there is potentially 1 in 5 people in the general population that have some form of neurodevelopmental disorder. With a prevalence so high, it may be time, from an evolutionary perspective, to stop talking about these people as “disordered” or “with errors of genetics”, challenging the “deficit” model of functioning. This is even more striking on social aspects: 32 to 35% of entrepreneurs are affected by ADHD or dyslexia, yet the assumption remains in the general public that these people are seriously disabled or perform bad in school. It is thus essential to be careful about the conversation and the language we’re using, so that these disorders are better understood by the general public, making diagnosis and treatments more accessible.
With neurodevelopmental disorders affecting the daily life, it is crucial for better disease management to consider interventions out of the clinical setting. Challenging this widespread vision of neurodevelopmental psychological disorders, information dissemination should be favoured so that the precious advances from the scientific population and clinical research reach families, education workers and family doctors. It appears to be an important lever to improve the outcomes for the population with neurodevelopmental disorders.

Intervention on neurodevelopmental disorders

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DESIRE European project

There are several key principles to keep in mind to insure that interventions on neurodevelopmental disorders are made as efficient as possible. First, these interventions must start early enough in the disease history, thus relying heavily on sensitivity and specificity of diagnostic approaches such as neuroimaging, neurophysiological tests, clinical evaluation and genetic tests. Early and specific diagnosis allows for earlier interventions that can not only be tailored for a specific patient but also developed as intensive interventions, which is a second key principle. Indeed, multi-axial interventions that involve parents and caretakers are transferred to daily life, amplifying the intervention time.
The supposed influence of natural history of a disease process that supposedly interferes with brain plasticity, and environmental influences are also to consider in interventions. Additionally, the identification of a growing number of gene abnormalities in the recent years has prompted initiatives in terms of precision medicine.

Evolutions in medication development, however, have been disappointing in the recent years, as illustrated with the example of epilepsy. Even though a considerable number of genes have been identified in the last decade for epileptic encephalopathies with severe neurodevelopmental disabilities, the increase in the number of medication studied has been slow and with little or absent mechanism-of-disease based development programs. With newly produced drugs being mostly targeted for epilepsy at large, which guarantees a broader market, we can see how long the way is from gene discovery to precision medicine.
In contrast, new pharmacological interventions are investigated when specific biological pathways are identified to be of interested in a particular disorder. This is the case, for example, with the identification of somatic mutations in the brain causing a dysregulation in the mTOR pathway, thus supposedly leading to brain malformations and namely hemimegalencephaly (Lee et al., Nature Genetics, 2012). Following a first study in rodents that tackled this increased expression of mTOR with Rapamycin (Lim et al., Nat Med, 2015), a few mTOR-related epilepsy trials have been launched, with some therapeutic successes (namely on tuberous sclerosis, a complex neurodevelopmental disorder). Three other trials are worth mentioning, that target neurobiological mechanisms in PCDH19, CDKL5 and SCN1A (ion channels) potentially related to some disorders (out of 1137 trials on ‘epilepsy’ registered on ClinicalTrials.gov as of August 2018). For instance, observations derived from experiments in Duchenne muscular dystrophy, cystic fibrosis and some lysosomal disorders have demonstrated that Ataluren allows to bypass impaired transcription resulting from nonsense mutations, which might prove beneficial for some patients suffering from CDKL5-related disorders and other encephalopathies.

Intervention on autism spectrum disorders, on the other hand, gives an example of how diverse the approaches can be to address some neurodevelopmental disorders. Medication-based interventions are often considered non- specific for the characteristics of autism, even though they can help improve some aspects of the disorder. Thus, behavioural and communication approaches are generally favoured in autism spectrum disorders, targeting organization in child’s behaviour and everyday life for a better efficiency. Dietary approaches have also been proposed, yet with disappointing results, as well as complementary and alternative treatments that fall outside of the typical medical recommendations. Among the 1252 trials registered for ‘autism’ on ClinicalTrials.gov in April 2020, most were either based on behavioural techniques,

devices-assisting technologies, biofeedback, diagnostic telemedicine, or parent-mediated intervention involving play, language and engagement training.
There is a major methodological issue with non-pharmacological interventions, however, regarding outcome measures, as they rely on tests in children with scores provided by parents or caretakers. Little to no standardization nor unified recommendations based on control population exist for these tests which are largely affected by social environments and local translation. It is important to promote efforts to efficiently address this issue and make it easier to test outcome measures. There is an initiative worth mentioning in the US in this regard, that started from studies characterizing the histopathological structure of the brain in some categories of children with autism, and in particular with megalencephaly. In addition to identifying impairment of cortical lamination, these studies more importantly allowed to build brain banks for autism in the US. Another brain bank for epilepsy can also be mentioned in Europe, a collaborative study over different European countries that has been going for several years and collected almost 10,000 specimens from epilepsy surgery (Blumcke *et al.*, N Engl J Med, 2017). These brain banks have proved themselves to be very informative for the research community and, although it might be challenging for the European culture, it is important to encourage brain bank initiatives for autism and other neurodevelopmental disorders.

In conclusion, there are several future directions to focus on in order to improve the interventions on neurodevelopmental disorders. Firstly, it is important to develop further understanding of these disorders, namely on the role of environmental factors and the genome-connectome relation highlighted by recent large neuroimaging and genomics studies. The identification of gene abnormalities is also a crucial direction for research, leading the way toward precision medicine. Some methodological improvements should be encouraged, such as parallel studies for translational models (confirming anatomical and behavioural findings in different animal models before translation to human), as well as initiatives such as brain banks for neurodevelopmental disorders.
Increasing diagnostic sensitivity is another major direction to prioritize, and it is especially true for diagnostic approaches that are based on early genetic screening, as we expect nowadays to identify a disorder before it is manifested. Additionally, it is important to encourage cross-disciplinary integration to tackle comorbidities, as for example cognitive delay, behavioural disorders, autism spectrum disorders and TDAH often come together in various combinations. Lastly, parents and caretakers need to be recognized as essential actors in the intervention, and their involvement should be promoted and amplified.

Panel discussion with representatives of European patient's organizations: Harald Neerland for Autism Europe and Dr. Tony Lloyd for ADHD foundation

Moderated by Marlies Dorlöchter, Etienne Hirsch and Bernard Poulain

The concluding panel discussion provided insights from the patient community to put forward their priorities in research and care for neurodevelopmental disorders. Both patient representatives agreed that a prevalent issue exists in how neurodevelopmental disorders are considered by the research and clinical community and the language used. This indeed contributes to the negative connotation surrounding these disorders in the general population, both socially affecting the patients and impairing the proper access to diagnosis. Even though understanding the genetics, causes and interplays between environmental and genetic factors is important, an over reliance on genetics alone in medicine and scientific research tends to reinforce the "disease" model of neurodevelopmental conditions. The prevalence is now so high that around 1 out of 5 people in the population could have some form of neurodevelopmental disorders, and it was thus suggested to refine the language used so that these individuals should not be considered "diseased", from an evolutionary point of view, nor should the scientific community talk about "curing" or "eradicating" neurodevelopmental disorders. It was consented by both patient representatives that in the context of the call text and the current nomenclature in the field it is appropriate to use the term neurodevelopmental "disorder" rather than "disease".

In this new paradigm, it is key to ensure proper knowledge dissemination on these disorders to the general population, which is currently lacking. It appears that useful information from the scientific and clinical communities does not sufficiently reach the people that could act in the daily life of patients: families, school workers and primary care practitioners. It is important to foster the dialogue between these communities and to ensure proper information sharing. This should also concern decision makers, as there is still some kind of disconnection between the advances in the research community and what is actually happening in terms of policies. Work remains on implementing the potential benefits of research in the health care system.
More widespread information and less stigmatisation would allow for better and earlier identification of the disorders. This early detection is critical in neurodevelopmental disorders, and research efforts focusing on earlier diagnosis are key for the patient community. With a better understanding of the genetic background of the conditions and other factors that cause a child to have neurodevelopmental disorders, it would indeed allow for earlier diagnosis and, consequently, to start interventions at an earlier age. Strong evidence proves that the earlier the intervention, the better the outcomes for the patients as they are provided help to function better in the society.
While the diagnosis for these disorders is medical, the support for patients is predominantly educational. For this reason, different treatment approaches should be considered in this regard to first and foremost provide care for patients, rather than trying to cure the disorder, which would require involving a larger spectrum of professionals. This means developing interventions driven by improving education, mental health and employability of people with neurodevelopmental conditions, avoiding the loss of life chances that remains far too frequent. An issue remains however in defining the outcomes of such interventions.

This last point begins to touch on some ethical questions that were briefly raised. One of such issues was the question how clinician can improve the communication of information, e.g. in pre-natal diagnostics, where results prognosing a high likelihood for neurodevelopmental disorders can burden parents-to-be with a lot of pressure and insecurity. This relates to a more

general interrogation: How can information about neurodevelopmental disorders be better communicated to parents, carers and schools, the general public and policy makers, to raise awareness for the needs of the affected? This kind of questions need in-depth consideration and own research.

Annex: List of attendants

Speakers

Fiona Francis	INSERM UMR-S 1270, Fer à Moulin Institute, Sorbonne University, Paris, France
Pierre Gressens	Université de Paris, NeuroDiderot, Inserm, Paris, France; Centre for the Developing Brain, Saint Thomas' Hospital, King's College of London, UK
Renzo Guerrini	Neuroscience Department, Anna Meyer Children's Hospital, University of Florence, Italy; DESIRE European project
Wieland B. Huttner	Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany
Julia Jacobs	Departments of Pediatrics and Clinical Neurosciences, Alberta Children Hospital, University of Calgary, Canada; Department of Pediatric Neurology, University of Freiburg Medical Centre, Freiburg, Germany
Tony Lloyd	ADHD Foundation, Liverpool, UK
Harald Neerland	Autism Europe
Anita Rauch	Institute of Medical Genetic, University of Zurich, Switzerland

Guests

Paul Olivier	GIS Autisme, Inserm, France
Vicky Whittemore	National Institute of Health (NIH), USA

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